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An Assessment of Pasteurization Treatment of Water, Media, and Milk with Respect to *Bacillus* Spores†

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ABSTRACT

This study evaluated the ability of spore-forming *Bacillus* spp. to resist milk pasteurization conditions from 72 to 150°C. Spores from the avirulent surrogate Sterne strain of *Bacillus anthracis*, as well as a representative strain of a common milk contaminant that is also a pathogen, *Bacillus cereus* ATCC 9818, were heated at test temperatures for up to 90 min in dH_2O, brain heart infusion broth, or skim milk. In skim milk, characteristic log reductions (log CFU per milliliter) for *B. anthracis* spores were 0.45 after 90 min at 72°C, 0.39 after 90 min at 78°C, 8.10 after 60 min at 100°C, 7.74 after 2 min at 130°C, and 7.43 after 0.5 min at 150°C. Likewise, log reductions (log CFU per milliliter) for viable spores of *B. cereus* ATCC 9818 in skim milk were 0.39 after 90 min at 72°C, 0.21 after 60 min at 78°C, 7.62 after 60 min at 100°C, 7.37 after 2 min at 130°C, and 7.53 after 0.5 min at 150°C. No significant differences ($P < 0.05$) in thermal resistance were observed for comparisons of spores heated in dH_2O or brain heart infusion broth compared with results observed in skim milk for either strain tested. However, spores from both strains were highly resistant ($P < 0.05$) to the pasteurization temperatures tested. As such, pasteurization alone would not ensure complete inactivation of these spore-forming pathogens in dH_2O, synthetic media, or skim milk.

Strict regulations have been mandated for dairy products in the Pasteurized Milk Ordinance *(26)* regarding the use of heating conditions for increasing the safety of such products with respect to pathogenic microorganisms. A milk product is considered pasteurized when the heating of every particle comprising that product can be established with certainty to have attained the legal minimum of 72°C for 15 s *(26)*. Certain spore-forming and psychrotrophic *Bacillus* spp. can survive standard pasteurization conditions and have been associated with the spoilage of milk products. One of the major contributors to the spoilage of pasteurized milk is *Bacillus cereus (7)*. In addition, *B. cereus* can cause illness by growth in milk and dairy products followed by the production of one of two types of enterotoxins—emetic and diarrheal—but only the latter is a food safety concern in these products *(27)*. Silage is a vector for transport of *B. cereus* spores from the soil through the bovine gastrointestinal tract to fecal waste that can subsequently splash the udder, resulting in external contamination of the milk stream *(24)*. Should the spores survive pasteurization, and then germinate and outgrow to produce sufficient levels of enterotoxin, foodborne illness could result following consumption.

In an attempt to predict the survival potential of spores of *B. cereus*, few studies have assessed the effects of stabilizing additives used in the heating of milk products *(12)*. Spores of *B. cereus* ATCC 9818 were less heat resistant in cream containing 20% fat (D_{98}-value of 6.10 min, where 98 is the temperature [°C]) compared with skim milk (D_{98}-value of 9.37 min) *(12)*. This pasteurization survival is of concern because endogenous *B. cereus* spores have been shown to reach cell densities of 10^5 CFU/ml in milk after 8 to 24 h at ambient temperatures. To make matters worse, such levels are also associated with harmful enterotoxin concentrations *(7)*.

Other spore-forming bacilli have also drawn much attention recently. The animal and human pathogen *Bacillus anthracis* is capable of causing intestinal anthrax, which although rare, can be lethal on ingestion. The majority of cases of intestinal anthrax have occurred in Africa, the Middle East, and Central and Southeastern Asia, having been attributed to insufficiently cooked meat contaminated with *B. anthracis* spores *(9)*. According to the Australia New Zealand Food Authority *(2)*, there have been no documented reports of anthrax ever being transmitted to humans through the consumption of milk or dairy products *(8)*. Nevertheless, few studies have examined the ability of *B. anthracis* spores to withstand pasteurization. In raw milk, *B. anthracis* spore levels (6 log CFU/ml) held constant for 48 h at temperatures from 5 to 37°C, whereas in pasteurized milk, spores germinated and grew to 8 log CFU/ml in 24 h at 37°C because of the heat shock of pasteurization. Although spores germinated in pasteurized milk, at 22°C, viability declined to 5 log CFU/ml after 4 h *(4)*. Pasteurization is believed to have caused the spores to germinate and outgrow to vegetative cells.

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In contrast, vegetative cells of *B. anthracis* decreased 4.5 log CFU/ml in 7 h at 37°C and 5.0 log CFU/ml in 24 h at 22°C in unpasteurized milk and decreased 4.5 log CFU/ml in 4 h at both 37 and 22°C when added to pasteurized milk *(4). B. anthracis* vegetative cells died at 5 to 22°C, decreasing 2.0 log CFU/ml after 24 h, but at 37°C, they grew 1 log CFU/ml over the same time period *(4).* Comparatively, vegetative cells of *B. cereus* naturally present in milk grew from unreported initial levels to greater than 6.0 log CFU/ml at 37°C and to greater than 5.0 log CFU/ml at 22°C in 24 h *(4).*

In another study, milk pasteurization at 63°C for 30 min or 72°C for 15 s inactivated about 4.0 log cells per ml of vegetative *B. anthracis* cells inoculated into raw milk but had no effect on the approximately 5 log spores per ml *(19).* A second pasteurization treatment (63°C for 30 min or 72°C for 15 s) 24 h after the first treatment resulted in spore germination and decreased germinated spores by 2 log CFU/ml, but still did not inactivate spores that did not germinate *(19).* Together, these data show that some pasteurization times and temperatures have little effect on the viability of *B. anthracis* spores.

The literature is replete with heat inactivation data on *B. anthracis* spores; however, differences in heat application (boiling water, steam, or dry heat), heating medium (physiological saline or dH_2O), strains tested (from blood swabs of different infected animals to isolates from different culture repositories), inocula levels (from 3.8 to 8.9 log spores), or a combination of factors limit the usefulness of these data for direct comparisons *(15, 22, 23).* Moreover, these previous reports cannot predict the heat resistance of *B. anthracis* spores in a complex food such as milk.

In this study, we examined the thermal stability of spores from *B. cereus* and *B. anthracis. B. cereus* ATCC 9818 was chosen because of its high heat resistance properties and because it was a commercially available spore former that was isolated from milk in 1952 *(11, 12).* Likewise, the Sterne strain of *B. anthracis* was selected as an ideal surrogate strain because it is indistinguishable from the virulent Ames strain of *B. anthracis,* except for the absence of plasmid pXO2, which encodes for the capsular protein genes necessary for virulence *(25).* In addition to skim milk, we also evaluated brain heart infusion (BHI) broth and dH_2O with respect to pasteurization efficiency on spore inactivation. Skim milk was used as the model heating medium containing dairy constituents. The BHI broth was included as a typical complex microbiological medium available to most research laboratories. Distilled water was incorporated to address the potential for protective effects afforded by the other more complex heating menstra. This is the first study to test the limitations of a range of established pasteurization temperature-time conditions with respect to spore-forming organisms of concern in milk.

MATERIALS AND METHODS

Microorganisms. Spores of the Sterne strain of *B. anthracis* were obtained from Drs. Jeff Karns and Michael Perdue (U.S. Department of Agriculture, Agricultural Research Service, Beltsville Area Research Center, Beltsville, Md.). *B. cereus* ATCC 9818 was purchased from the American Type Culture Collection (Manassas, Va.).

Spore preparations. Single colonies were selected from BHI (Difco, Becton Dickinson, Sparks, Md.) agar plates previously incubated at 37°C overnight. With the aid of an inoculation loop, colonies were transferred to 10 ml of BHI broth (pH 6 to 7 at 25°C). The tubes were incubated at 37°C with shaking (200 rpm) for about 18 h. Cultures were then diluted in 0.1% (wt/vol) peptone water and spread plated onto new sporulation medium *(19)* for *B. anthracis* or onto nutrient agar (Difco, Becton Dickinson) with 3 mg/liter manganese sulfate (Fisher Scientific, Pittsburgh, Pa.) for *B. cereus (11).* Plates were incubated at 37°C for 72 h. Sporulation levels were checked daily with an Olympus model BH-2 microscope equipped with phase contrast optics (Olympus, Melville, N.Y.) until 90% sporulation was achieved. Two milliliters of sterile dH_2O was added to each of 25 plates, and spores were harvested with sterile L spreaders (Daigger and Co., Inc., Vernon Hills, Ill.). Spores were harvested from 25 plates, washed twice with 1 ml of dH_2O, pooled, pelleted ($5{,}000 \times g$ for 20 min), and resuspended in dH_2O to a volume of 40 ml. Ethanol was added to 20% (vol/vol), and spore stocks were stored at 4°C until used. Spore titers were determined by plating serial dilutions of spore stocks, with and without prior heat shock (75°C for 20 min in dH_2O), onto BHI agar plates. After the plates were incubated overnight at 37°C, data were collected (CFU per milliliter).

Simulated pasteurization conditions for testing spore resistance. For the lower temperatures (72 and 78°C), the heating media consisted of 150 ml of dH_2O, BHI broth, or skim milk (pH 6.5 at 25°C) pre-equilibrated to 72 or 78°C, to which 3 ml of the spore suspension ($\sim 10^{10}$ spores per ml) was then added. The mixture ($\sim 10^{8}$ spores per ml) was aseptically stirred in an Exacal high-temperature water bath (model EX-251-HT, Neslab Instruments, Inc., Portsmouth, N.H.) with a submersible magnetic stirrer (model 230, VWR Scientific Products, West Chester, Pa.). Samples were taken at preselected time intervals, extending up to 90 min, serially diluted in 0.1% (wt/vol) peptone water, and plated onto BHI agar base medium (Difco, Becton Dickinson) with a spiral plater (autoplate 4000 model D, Spiral Biotech, Bethesda, Md.). Plates were incubated aerobically at 37°C for 16 to 20 h, and spores were counted (CFU per milliliter) for each time point. Background flora was measured at approximately 10^3 to 10^4 CFU/ml and was well below the level of spore inoculation.

For the higher pasteurization temperatures (100, 130, and 150°C), spore suspensions (2 ml) were mixed with 18 ml of dH_2O, BHI broth, or skim milk (pH 6.5) and dispensed into sterile ½-dram (2 ml) glass sample vials (12 by 38 mm) with rubber-lined caps to exclude all headspace (Wheaton Science Products, Millville, N.J.). Come-up times (CUTs) were predetermined in triplicate in control vials with an Atkins thermocouple thermometer (series 396, Atkins Technical, Inc., Gainesville, Fla.) submerged in a Polystat heated circulating bath (model EW-12105-30, Cole-Parmer Instrument Co., Vernon Hills, Ill.) containing silicone oil (The Chemistry Store.Com, Inc., Pompano Beach, Fla.). The CUTs were then used to establish temperature equilibration times for the spore suspensions ($\sim 10^{9}$ spores per ml) in the experimental vials for each heating medium. The vials were completely submerged under the oil for the CUTs until the desired starting temperatures were achieved and before collection of time-dependent heat inactivation data. Vials were removed at the selected exposure intervals, cooled on ice, and immediately diluted in 0.1% (wt/vol) peptone water. Samples were plated on BHI agar base medium with a spiral plater. Plates were incubated aerobically at 37°C for 16 to 20 h before colonies were counted.

TABLE 1. *United States time and temperature parameters for pasteurization of milk*

Temp (°C/°F)	Time	Designation	Reference
63/145	30 min	Low temp–long time	*26*
72/161	15 s	High temp–short time	*26*
89/191	1.0 s		*26*
90/194	0.5 s		*26*
94/201	0.1 s		*26*
96/204	0.05 s		*26*
100/212	0.01 s		*26*
138/281	At least 2 s	Ultrapasteurization	*5*
138–150/302	1 or 2 s and aseptic packaging	Ultrahigh temp	*5*

Calculation of *D*-values. The D_x-values (time [min] at 72, 78, 100, 130, or 150°C required to reduce the viable cell population by 90%) were determined by separately plotting the log number of survivors against time at each temperature with Microsoft Excel 2000 software (Microsoft Corporation, Redmond, Wash.). The best-fit line for survivor plots was determined by regression analysis *(16).* A regression equation of the type $y = a + bx$ was derived, where b is the slope of the best straight line that, when inverted and changed from negative to positive, gives the D_x-value for a specific temperature (x). Only survivor curves with five or more values in the straight portion were used. z-values were determined from the best-fit line for *D*-values over the previously mentioned experimental temperatures.

Statistical analyses. Each spore batch for a strain was tested at each of the five temperatures in each of three separate trials with duplicate samples at each time interval. Means (± standard deviations) were calculated from individual sets of data for each trial. The SAS system *(20)* was used to determine statistically significant differences among treatments. The Bonferroni mean separation test was used to determine significant differences ($P < 0.05$) among means *(14).* An analysis of covariance was used to determine the effect of medium and strain on the regressions of log (*D*-value) against temperature *(16).*

RESULTS AND DISCUSSION

The purpose of this study was to examine the effectiveness of time and temperature parameters practiced in the pasteurization of milk products against *B. anthracis* Sterne and *B. cereus* ATCC 9818. Previous studies described spore inactivation times and temperatures in dilute aqueous media *(22)* or inactivation times and temperatures in milk at the lower temperature limits of pasteurization *(4, 12, 19).* This is the first study to comparatively address the higher temperature limits of milk pasteurization with respect to spores of *B. cereus* ATCC 9818 and *B. anthracis* Sterne. As detailed below, the most significant finding of this study is that higher heating temperatures and longer exposure times are needed compared with those reported to inactivate spores of *B. anthracis* Sterne and *B. cereus* ATCC 9818.

Time and temperature requirements approved for pasteurization of milk are listed in Table 1. The recommended processing guidelines are determined by the quality attributes of a specific milk product *(26).* In general, as the pasteurization temperature is increased, exposure times are decreased to prevent excessive degradation of milk constituent proteins. Temperatures of 72 or 78°C were chosen in this study to evaluate standard milk pasteurization parameters, whereas higher temperatures, as used in ultrapasteurization and ultrahigh-temperature (UHT) pasteurization, were selected because of the thermal stability of *B. cereus* and *B. anthracis* spores.

The temperatures of 72 or 78°C, even beyond recommended treatment times of 15 to 30 s, did not effectively inactivate the spores of *B. cereus* ATCC 9818 or *B. anthracis* Sterne (Fig. 1 and Table 2). Even after 90 min, less than a 1-log decrease in viable spores was observed. Moreover, these lower temperatures produced considerable variations in *D*-values (ca. ±100%), resulting in no more than a 1-log reduction (0.12 ± 0.35 to 1.08 ± 0.50). The theoretical times to a 12-*D* reduction (i.e., commercial sterility) at 72 and 78°C were estimated at 380 to 14,263 min, which were well above the 15-s exposure limit at 72°C or even the 30-min exposure at 63°C (Table 2). The R^2 values obtained from the best-fit linear adaptations for spore thermal inactivation and *D*-value calculation studies indicated that the first-order model representations for heat inactivation did not apply to the spore populations in this study (Table 2). Decimal reduction times or *D*-values calculated at 72, 78, 100, 130, and 150°C for *B. anthracis* and *B. cereus* spores were considered invalid because of low R^2 values obtained for linear representations of collected data points (Table 2). For this reason, the thermal death curves depicted in Figure 1 were more useful for comparing results. Shoulders and tails for thermal inactivation curves are expected observations for spore populations *(11).* Some survival curves for spores at a heating temperature of 100°C showed a rapid initial drop in percent survival, down to 10% in the first 5 min, followed by a steady rate of decline or plateau indicative of a heterogeneous population of cells (Fig. 1). Although the initial rapid rate decrease might be explained by inactivation of vegetative cells within the spore preparations, microscopic evaluations confirmed that over 90% of the total cell population was phase bright spores (data not shown). At the same time, 90% of the *B. anthracis* spore population exhibited inactivation within 5 min at 100°C in BHI broth (Fig. 1C). The remaining spores were not killed even after an additional 25 min of heat treatment (Fig. 1C). A similar trend was exhibited when spores were heated to 100°C in dH_2O (Fig. 1E and 1F). Spores from a single strain characteristically show different environmentally induced resistance characteristics by the dipicolinic acid content, temperature of sporulation, degree of hydration, and mineral content *(1, 3, 6, 10, 13, 18, 28).* It is not beyond reasonable expectations to assume that a single batch of spores might have cells that exhibit heterogeneous properties with respect to germination, survival, or both traits. Previous attempts have been made with varying degrees of success to synchronize populations of spores to avoid such anomalous deviations *(27).* Total 100% syn-

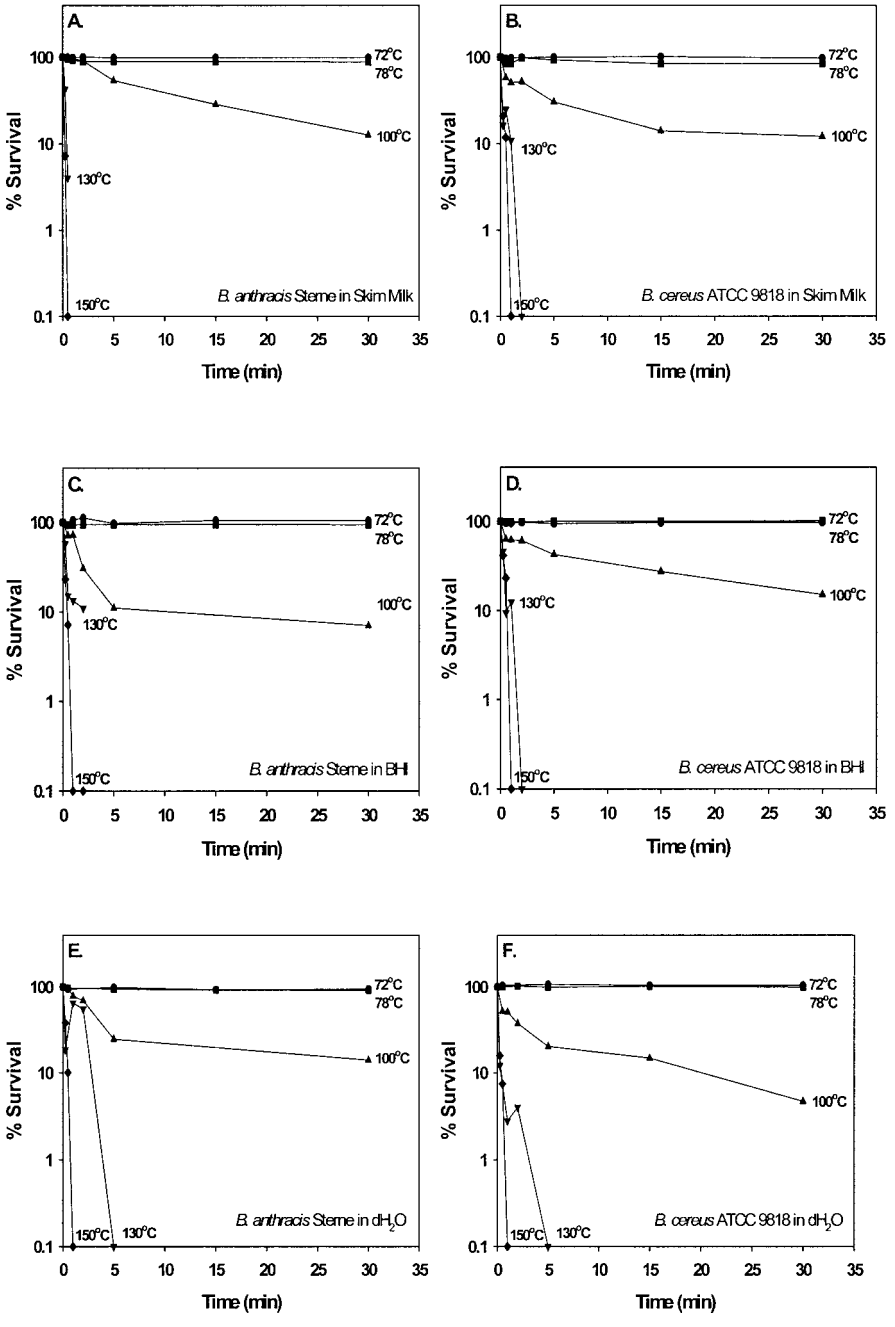

FIGURE 1. B. anthracis *Sterne (A, C, E) and* B. cereus *ATCC 9818 (B, D, F) spores heat killed in skim milk (A, B), BHI broth (C, D), and dH_2O (E, F) over time. Spore survival at 72°C (●), 78°C (■), 100°C (▲), 130°C (▼), and 150°C (♦).*

chrony of spores has never been achieved and was not attempted here.

In this study, viable spore counts increased less than 1 log CFU/ml following heat treatment for less than 10 min at temperatures of 100, 78, and 72°C compared with untreated spores (data points >100% survival for Fig. 1). Often, such data points, although characteristic for spores, are deleted as ''outliers'' to accommodate linear curve fitting programs such as those in SAS (SAS 1989). These findings certainly contributed to the large variations experienced for the *D*-values calculated for spores from *B. anthracis* Sterne and *B. cereus* ATCC 9818 exposed to 72 and 78°C (Table 2). The greatest log reduction at 72 or 78°C was only 1.08 log CFU/ml after 90 min, and there were no differences among dH_2O, BHI broth, and skim milk in spore lethality. Under all conditions tested for 72 and 78°C for dH_2O, BHI broth, and skim milk, the log reductions indicated that these temperatures were too low to be effective pasteurization requirements for inactivating spores of *B. anthracis* Sterne or *B. cereus* ATCC 9818. At 100, 130, and 150°C, more than 7 log reductions were obtained, but only after 60, 5, and 1 min, respectively. Such extended times at these higher temperatures caused deleterious effects on the milk constituents.

The CUTs at 100, 130, and 150°C were 9.5, 8.9, and 5.5 min, respectively, for dH_2O; 10.0, 8.0, and 5.7 min, respectively, for BHI broth; and 9.0, 7.5, and 5.0 min, respectively, for skim milk. Unfortunately, even though CUTs were subtracted from total treatment times, the effects on spores during the 5- to 10-min CUT have not been fully evaluated. The long CUTs were necessary to ensure that treatment temperatures were attained in the middle (coolest)

TABLE 2. *Calculated thermal resistance parameters for spores*

Strain	Temp (°C)	Medium	*D*-value (min)[a]	Theoretical inactivation (min)[b]	*z*-Value (°C)	Maximum experimental log reduction	Time to maximum measured reduction (min)[c]
B. anthracis Sterne	72	dH_2O	875.7 ± 981.2 AB (R^2 = 0.2599)	10,508	22.1 (R^2 = 0.968)	0.53 ± 0.81	90
		BHI	612.9 ± 431.5 AB (R^2 = 0.1297	7,355	21.7 (R^2 = 0.987)	0.38 ± 0.54	
		Skim milk	1,188.6 ± 775.6 (R^2 = 0.2869)	14,263	20.4 (R^2 = 0.952)	0.45 ± 0.59	
	78	dH_2O	115.4 ± 32.3 B (R^2 = 0.904)	1,385	22.1 (R^2 = 0.968)	1.08 ± 0.50	90
		BHI	351.5 ± 322.8 AB (R^2 = 0.129)	4,218	21.7 (R^2 = 0.987)	0.37 ± 0.30	
		Skim milk	513.3 ± 640.3 AB (R^2 = 0.306)	6,160	20.4 (R^2 = 0.952)	0.39 ± 0.40	
	100	dH_2O	10.9 ± 1.2 C (R^2 = 0.610)	130.7	22.1 (R^2 = 0.968)	7.78 ± 0.50	60
		BHI	13.1 ± 2.2 C (R^2 = 0.434)	156.8	21.7 (R^2 = 0.987)	6.92 ± 0.43	
		Skim milk	7.1 ± 0.6 C (R^2 = 0.854)	85.2	20.4 (R^2 = 0.952)	8.10 ± 0.17	
	130	dH_2O	0.69 ± 0.17 DEF (R^2 = 0.916)	8.3	22.1 (R^2 = 0.968)	7.54 ± 1.15	2–5
		BHI	0.92 ± 0.27 DE (R^2 = 0.644)	11.0	21.7 (R^2 = 0.987)	7.86 ± 0.45	
		Skim milk	1.12 ± 0.10 D (R^2 = 0.305)	13.4	20.4 (R^2 = 0.952)	7.74 ± 0.26	
	150	dH_2O	0.16 ± 0.00 F (R^2 = 0.886)	1.9	22.1 (R^2 = 0.968)	7.66 ± 0.14	0.5–1
		BHI	0.17 ± 0.04 F (R^2 = 0.893)	2.0	21.7 (R^2 = 0.987)	7.40 ± 0.80	
		Skim milk	0.19 ± 0.01 EF (R^2 = 1.000)	2.3	20.4 (R^2 = 0.952)	7.43 ± 0.65	
B. cereus ATCC 9818	72	dH_2O	94.3 ± 35.10 B (R^2 = 0.801)	1,132	36.5 (R^2 = 0.970)	0.43 ± 0.23	90
		BHI	469.0 ± 238.5 A (R^2 = 0.034)	5,628	21.7 (R^2 = 0.979)	0.12 ± 0.35	
		Skim milk	246.1 ± 83.6 AB (R^2 = 0.482)	2,953	26.9 (R^2 = 0.985)	0.39 ± 0.56	
	78	dH_2O	31.7 ± 3.6 BC (R^2 = 0.405)	380	36.5 (R^2 = 0.970)	0.15 ± 0.69	60
		BHI	464.9 ± 86.0 A (R^2 = 0.373)	5,579	21.7 (R^2 = 0.979)	0.13 ± 0.17	
		Skim milk	80.6 ± 20.9 B (R^2 = 0.178)	967	26.9 (R^2 = 0.985)	0.21 ± 0.34	
	100	dH_2O	11.5 ± 1.3 C (R^2 = 0.448)	138	36.5 (R^2 = 0.970)	7.97 ± 0.62	60
		BHI	11.1 ± 3.4 C (R^2 = 0,640)	133	21.7 (R^2 = 0.979)	7.44 ± 1.34	
		Skim milk	12.2 ± 2.2 C (R^2 = 0.498)	146	26.9 (R^2 = 0.985)	7.62 ± 1.11	
	130	dH_2O	1.81 ± 1.03 D (R^2 = 0.293)	21.7	36.5 (R^2 = 0.970)	7.93 ± 0.56	2–5
		BHI	0.99 ± 0.18 DE (R^2 = 0.423)	11.9	21.7 (R^2 = 0.979)	8.75 ± 0.38	
		Skim milk	1.96 ± 1.75 D (R^2 = 0.526)	23.5	26.9 (R^2 = 0.985)	7.37 ± 1.52	
	150	dH_2O	0.16 ± 0.00 F (R^2 = 0.886)	1.9	22.1 (R^2 = 0.968)	7.66 ± 0.14	0.5–1
		BHI	0.17 ± 0.04 F (R^2 = 0.893)	2.0	21.7 (R^2 = 0.987)	7.40 ± 0.80	
		Skim milk	0.19 ± 0.01 EF (R^2 = 1.000)	2.3	20.4 (R^2 = 0.952)	7.43 ± 0.65	

[a] Values given are mean ± standard deviation (average of three independent experiments run in duplicate), with the same letter indicating no statistically significant differences ($P < 0.05$).

[b] 12-*D* reduction (commercial sterility).

[c] Plating detection limits were 10 to 20 CFU/ml, and surviving cells below this concentration might not have been recorded.

portion of the sample vials. Obviously, not all spores could be expected, within even a single sample, to reach test temperatures at an exact time point, but deviations were kept to a minimum. Significant differences in lethality of spores were not found among skim milk, dH_2O, and BHI broth or between *B. anthracis* Sterne and *B. cereus* ATCC 9818 at the temperatures evaluated (Table 2). Following an analysis of covariance, it was concluded that differences in medium composition did not contribute to any evidence of slope or quadratic coefficient variations. Despite low R^2 values, estimated times to a 12-*D* reduction of spores decreased by a factor of 10 at each successive temperature level from 72 to 78 to 100 to 130 to 150°C. The log reduction ranged from 6.92 ± 0.43 to 8.10 ± 0.17 for *B. anthracis* spores and from 7.44 ± 1.34 to 8.75 ± 0.38 for *B. cereus* spores at 100°C in 60 min to 150°C in 1 min, respectively.

The *D*-values obtained from this study for spores of *B. anthracis* Sterne were higher than the 10 min needed to completely inactivate 10^6 spores at 100°C as reported in the literature for spores from other strains of *B. anthracis (15).* An emphasis has been placed on the total spore reductions in this study because R^2 values for the plotted data suggest that calculated *D*-values do not appropriately reflect the characteristics of spore populations in a linear manner under the tested pasteurization conditions (Table 2). Other studies indicated that spores of *B. anthracis* at levels of 10^8 spores per ml could be effectively inactivated at 100°C within 5 to 10 min *(15, 21).* Differences among studies could be explained, at least in part, by differences in the levels of spores (3.8 to 8.9 log spores), the percentage of spores versus vegetative cells (~90% in this study), the medium in which spores were stored (bloody swabs of animal carcasses or dH_2O), CUT (ranging from 5 to 10 min in this study), or a combination of factors *(29).* Clearly, spore preparations in the previous studies could have included higher percentages of vegetative cells, storage differences for spore preparations (physiologic solutions of sodium chloride), or even different time and temperature conditions for the pasteurization of spores than those used in this study. Results from this study suggest that if spores were inoculated to 12 log spores per ml, it would require 130.7 min in dH_2O, 156.8 min in BHI broth, and 85.2 min in skim milk for complete inactivation (12-*D* reduction) at 100°C (Table 2). Because of protocol differences among studies, data obtained for treatments other than at 100°C were not valid for comparison among the available literature. The values obtained in this study are considerably greater than values for 12-*D* reductions determined in earlier studies and accentuate the differences among experimental conditions used for measurement and for verification of safe food processing requirements.

The D_{100}-values obtained in the present study for *B. cereus* ATCC 9818 spores at 100°C in dH_2O, BHI broth, and skim milk were 11.5 ± 1.3, 11.1 ± 3.4, and 12.2 ± 2.2 min, respectively (Table 2). Although the appropriateness of using these *D*-values is questioned because of the low R^2 values, these values agreed more closely with previous studies using this strain. For example, Mazas et al. *(12)* reported D_{98}-values of 9.37 for skim milk at pH 5.6 to 5.7. The observed subtle differences might be attributed to the slightly higher pH of the commercial skim milk used in this study (i.e., pH 6.5); acidic pH might lower the heat resistance properties of microorganisms *(11, 17).* In a similar study with spores from *B. cereus* ATCC 9818, D_{103}-values in McIlvain buffer at pH 4.0 were 0.47 ± 0.03 min compared with 4.76 ± 0.38 min in pH 7.0 buffer *(11).*

At UHT pasteurization temperatures (Table 1), a maximum 7.7-log reduction for *B. anthracis* Sterne spores and 7.6-log reduction for *B. cereus* ATCC 9818 spores was attained in 0.5 to 1.0 min at 150°C. Times of 1.9 to 2.3 min were calculated for 12-*D* reductions of *B. anthracis* Sterne spores and 1.9 to 2.2 min for *B. cereus* ATCC 9818 spores at these same UHT conditions (Table 2). These exposure times are well above the 2-s time restriction for pasteurization of milk products at UHT as required by the U.S. Food and Drug Administration Pasteurized Milk Ordinance *(26).*

The *z*-value is the temperature change required to alter the *D*-value by a factor of 10, and it reflects the temperature dependence of the heat inactivation reaction. A higher *z*-value would indicate greater heat resistance of the spores. The *z*-values derived in this study (Table 2), although dependent on *D*-values with rather low R^2 values, were independent of the heating medium. In general, the rate of heat transfer will differ among media because of their respective heat capacities or composition. It was suggested that the thermal death mechanism of spores from *B. cereus* ATCC 9818 could be different in an acidic medium compared with a neutral pH medium, and the same could be expected for *B. anthracis (11).* Overall, the spores of *B. cereus* ATCC 9818 were comparable to and not significantly different ($P < 0.05$) from spores of *B. anthracis* Sterne with regard to heat resistance in dH_2O, BHI broth, and skim milk, regardless of temperature, on the basis of percent survival, *D*-value, and *z*-value calculations.

Although spores can survive pasteurization treatments, they are considered metabolically dormant. Only in favorable growth temperatures (ambient temperature to 37°C) would the spores be expected to germinate. Even so, our data indicate that vegetative cells of *B. anthracis* could be expected to die in milk should they germinate, as opposed to vegetative cells of *B. cereus,* which could potentially increase in cell count *(4).* It also is somewhat reassuring that under natural conditions, only *B. cereus* would be found in milk products, as opposed to *B. anthracis (2, 7, 8).*

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